Microbial Burden Assessment of Commercial Aircraft Cabin Air

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ABSTRACT

The microbial burdens of 69 cabin air samples collected from commercial airliners were assessed via conventional culture-dependent, and molecular-based microbial enumeration assays. Cabin air samples from each of four separate flights aboard two different carriers were collected via air-impingement. Microbial enumeration techniques targeting DNA, ATP, and endotoxin were employed to estimate total microbial burden. The total viable microbial population ranged from 0 to 3.6 x 10^4 cells per 100 liters of air, as assessed by the ATP-assay. When these same samples were plated on R2A minimal medium, anywhere from 2% to 80% of these viable populations were cultivable. Five of the 29 samples examined exhibited higher cultivable counts than ATP-derived viable counts, perhaps a consequence of the dormant nature (and thus lower concentration of intracellular ATP) of cells inhabiting these air cabin samples. Ribosomal RNA gene sequence analysis showed these samples to consist of a moderately diverse group of bacteria, including human pathogens. Enumeration of ribosomal genes via quantitative-PCR indicated that population densities ranged from 5 x 10^1 to 10^7 cells per 100 liters of air. Each of the aforementioned strategies for assessing overall microbial burden has its strengths and weaknesses; this publication serves as a testament to the power of their use in concert.

INTRODUCTION

Studies of aircraft cabin air have been conducted for a number of years (Nagda, 2000; 2001) and have concentrated on chemical factors related to human health; ozone, carbon dioxide, temperature, air flow volumes, and volatile organic carbons typically head the list. Under a Federal Aviation Administration program, a recent ASHRAE (American Society of Heating, Refrigeration and Air Conditioning Engineers) study sampled on-board followed by off-line chemical analysis (Spicer, C., unpublished data). The FAA has begun to emphasize the significance of microbial air contamination circulating throughout the cabins of commercial airliners. Microorganisms are ubiquitous throughout the cabin air systems, and passengers and crewmembers are thought to be the predominant source of microbial contamination. Since the presence of viable microorganisms circulating about cabin air is of particular consequence to human health, it is critical to thoroughly and accurately assess the overall microbial burden associated with commercial cabin air systems. While the microbial burden associated with cabin air is the focus of this study, it is but one of many initiatives in place to minimize the likelihood of acquiring air-travel-associated illness. The Environmental Protection Agency recently advised air passengers with compromised immune systems to ask for canned or bottled beverages and to refrain from drinking tea or coffee unless made with bottled water (The Associated Press; Jan. 19, 2005).

To date, every attempt at monitoring the microbial diversity aboard commercial airliners has relied on traditional, cultivation-dependent approaches. Such techniques are fraught with bias as different species require different media or conditions for growth and only a small percentage of bacteria in any given environment are capable of being cultivated; therefore, in current microbial quality verification protocols not all cells are likely to be detected as colony-forming units (CFU) (Amann et al., 1995). In this study, we utilize both conventional culture-based and the state-of-the-art molecular techniques, including ATP, DNA, and LPS-based assays, to elucidate the overall microbial burden present in the cabins of commercial aircraft. Cabin air samples were spatially and temporally collected via impingement aboard 4 commercial airline flights. For each flight, samples were collected prior to boarding, upon reaching cruise altitude, mid-flight, prior to descent, and post-boarding from each of the following locations: seat, floor, gasper, and lavatory. For each sample 750 L of air, the amount an average human exchanges every 2 hours, was impinged into 5mL sterile buffer.

Adenosine Tri-Phosphate (ATP) is an energy carrier used by all living organisms that can be exploited as an indicator of the presence of living organisms, as many earlier studies have suggested (Venkateswaran et al., 2002; Chappelle and Lewin 1968; Karl, 1980; Stanley 1989). In theory, the ATP-based assay detects all cells, regardless of cultivability, and can differentiate viable from dead microbes via the enzymatic removal of exogenous ATP prior to quantification (Venkateswaran et al., 2002, Pace et al., 1985, Giovannoni et al., 1990). We have recently utilized molecular-based techniques in estimating the microbial burden of ISS drinking water and have shown the presence of DNA sequences related to human pathogens (La Duc et al., 2003). Here, we em-

ploy such methods in a thorough, polyphasic assessment of the microbial burden associated with commercial airliner cabin airs. This study encompasses several samplings of cabin air aboard two independent, commercial carriers, referred to herein as airline A and airline B.

MATERIALS and METHODS

Sample collection. Cabin air samples were spatially and temporally collected via impingement aboard 4 commercial airline flights. For each flight, samples were collected prior to boarding, upon reaching cruise altitude, mid-flight, prior to descent, and post-boarding from each of the following locations: seat, floor, gasper, and lavatory. For each sample 750 L of air, the amount an average human exchanges every 2 hours, was impinged into 5mL sterile buffer with a Mesosystems BT-550 air impingement device. Immediately following collection each sample was placed on ice and transported to a -20°C freezer until further processing. All air samples were analyzed within 3 days of collection. This study focuses on ten samples, each of which arose from the pooling of ~ 5 individually collected air-impinged samples.

Cultivation procedures. One hundred microliter aliquots of each sample were placed into petri dishes and total aerobic counts were enumerated by the pour plate technique using minimal media (R2A agar; Difco Laboratories, Detroit, MI). Following the recommendations of the Space Study Board, colony-forming units (CFUs) of total heterotrophs were counted after incubation at 32°C for up to 7 days. Isolates were selected, purified, and stored at –80°C for further processing and analysis. Identification of purified strains was determined based on 16S rDNA sequencing (see below).

ATP-based bioluminescent assay. Firefly luciferase catalyzes the reaction of luciferin with ATP to form the intermediate luciferyl adenylate. Its subsequent reaction with oxygen leads to a cyclic peroxide, whose breakdown yields CO_2 plus the oxyluciferin product in the electronically excited state, from which a photon is emitted with a quantum yield of about 90% (Wilson and Hastings, 1998). The bioluminescence generated is directly proportional to the amount of ATP in the sample being assayed, and to the number of microorganisms in the sample assayed. In the kit used (see below), samples of 0.1 mL were assayed in a total volume of 0.3 mL, with one relative light unit corresponding to 2×10^{-14} M ATP. The dynamic range of the assay with such samples ranges from 5×10^{-12} M to 10^{-7} M ATP. The use of firefly luciferase to assay ATP extracted from microorganisms provides a straightforward means to enumerate microbes within minutes, and several companies have developed kits based on this system. For our purposes, these kits allow the detection of total ATP, not only the intracellular ATP from intact microbial cells, but also the extracellular ATP from dead bacteria and non-microbial sources, such as lysed cells or debris. The extracellular ATP can be eliminated enzymatically, so that the remaining ATP is attributable only to intact microbial cells, both cultivable and non-cultivable. A kit for this purpose (CheckLite-HS Set, Kikkoman Corp., San Francisco, CA) was used in the present study.

LPS-based endotoxin assay. When microorganisms invade an animal, the immune system responds by initiating a highly specific enzyme cascade in its blood cells (i.e. amebocytes). It is known that this cascade is initiated by the presence of lipopolysaccharide found in the outer membrane of Gram-negative bacteria and beta-glucan in yeasts, and results in the formation of a gel-clot that destroys invading microbes. The LPS-based microbial detection assay exploits this principle as it occurs in the horse-shoe crab (*Limulus polyphemus*) coupled with a chromogenic substrate. This method is most commonly used to quantify endotoxin and is the basis for the American Society for Testing and Materials method E2144-01. Commercially available kits (Charles River Laboratories, Wilmington, MA) were used to perform this rapid and simple assay (2-hour) where appropriate aliquots of samples were mixed with reaction reagents and clotting was measured colorimetrically.

DNA extraction and PCR amplification. DNA was extracted directly from each of the 69 individually collected samples. Approximately 10 mL from each pooled sample was concentrated and subjected to DNA extraction following standard lysozyme/organic solvent extraction protocols. In addition, DNA was extracted from purified isolates using a commercial kit (Promega, Madison, WI). Bacterial small subunit (SSU) rRNA genes were PCR-amplified with eubacterially biased primers B27F (5'-GAGTTTGATCMTGGCTCAG-3') and B1512R (5'-AAGGAGGTGATCCANCCRCA-3'). PCR conditions were as follows: 1 min. 95°C denaturation, 2 min. 55°C annealing, and 3 min. 72°C elongation for 35 cycles using a thermal cycler (MJ Research; Waltham, MA). After 10 minutes incubation at 72°C, the amplification product was purified with a gel excision kit (Qiagen, Chatsworth, CA). Purified amplicons were then fully, bi-directionally sequenced (Davis Sequencing, Davis, CA).

Sequence analysis and phylogenetics. The phylogenetic relationships of organisms covered in this study were determined by comparison of individual rRNA gene sequences to those in the public database (http://www.ncbi.nlm.nih.gov/blast). A maximum parsimony evolutionary tree encompassing 500 bootstrapped replicates was constructed using Phylogenetic Alignment Using Parsimony (PAUP) software (Swofford, 1990).

TaqMan Quantitative Polymerase Chain Reaction (Q-PCR). TaqMan Q-PCR reactions were performed in triplicate for each sample using an Applied Biosystems Inc. 7700 Sequence Detector. One microliter aliquots of extracted DNA from each sample served as template for amplification. Primers universal to known eubacteria, 1369F (CGGTGAATACGTTCYCGG) and 1492R (GGWTACCTTGTTACGACTT) (Suzuki et al., 2000) and the fluorescence-labeled probe TM1389F (CTTGTACACACCGCCCGTC), were used in this quantitative analysis. Each reaction mixture consisted of 25uL of 2X reaction mix (2X Taqman Universal PCR Master Mix, Applied Biosystems Inc, Foster City, CA), 0.8 uM of each oligonucleotide primer, 0.5 uM of the FAM/TAMRA-labeled oligonucleotide probe, and 1 uL template DNA in a final volume of 50 uL. Reaction conditions were as fol-

lows: 95°C denaturation for 10 min., followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, and elongation at 72 °C for 30 s.

RESULTS and DISCUSSION

Samples collected from flight #1 (Chicago to Seattle segment; Airline A), flight #2 (Seattle to Dallas FW; Airline A), flight #3 (Salt Lake City to Cincinnati; Airline B), and flight #4 (Cincinnati to Salt Lake City; Airline B) were subject to culture-independent, molecular-based microbial enumeration assays. In addition, samples collected aboard flights #3 and #4 of Airline B were also subject to culture-dependent plate count assays to assess total heterotrophic population diversity and to compare the efficacy of rapid molecular assays in the estimation of microbial burden. Results of these analyses are given in Table 1. Such comparisons will aid in the planning of future sampling events. Since the time between collection of samples and analysis might afford changes in the populations present in the samples, even when the best available preservation and sample transport technologies are exploited (von Wintzingerode F. et al., 1997), molecular assays able to analyze microbial burden rapidly in the field are of immense value.

For discussion purposes, the following terminologies are used as defined below. The values reported by the ATP assay are of two sorts, values arising from the total ATP-assay reflect the total microbial population while the intracellular ATP-assay reflects the viable portion of this population. Values reported from the LPS-based assay (picograms of LPS) are indicative of the total Gram-negative microbial burden. Finally, TaqMan Q-PCR values are indicative of all cells present (both dead and alive) and are expressed in terms of total ribosomal gene copy numbers (each cell bearing ~1 to 10 chromosomal rRNA gene copies).

Culture-independent analyses.

(i) ATP-based bioluminescent assay. Based on the results of the total ATP assay (accounting for both dead and live microbes) the total microbial burden across samples varied from 8.0×10^1 to 2.1×10^5 cells per 100 liters of air. Only one of the 69 samples analyzed failed to yield an ATP signal (Flight #1, post-deplane, lavatory). The undetectable ATP signal from this lavatory location, where a large microbial burden had been observed in all

Table 1. Microbial population[1] of various air samples collected from several locations of commercial airlines

Description of the samples	Total number of samples collected	ATP ($x10^4$ RLU)		Total cultivable population ($x10^4$ CFU) (C)	LPS ($x10^4$ pg endotoxin) (D)
		Total (A)	Intracellular (B)		
Flight #1					
Pre-Board	4	7.18	4.35	-[2]	0.07
Upon reaching cruise altitude	4	5.88	2.75	-	0.17
Half-way through flight	4	5.88	Undetectable	-	0.08
Just before beginning descent	4	1.67	Undetectable	-	0.05
After landing	4	0.48	Undetectable	-	0.08
Flight #2					
Pre-Board	4	1.38	Undetectable	-	0.09
Upon reaching cruise altitude	4	6.27	3.50	-	0.12
Half-way through flight	4	0.45	Undetectable	-	0.08
Just before beginning descent	4	1.97	Undetectable	-	0.07
After landing	3	1.18	Undetectable	-	0.09
Flight #3					
Pre-Board	4	2.85	0.95	0.21	0.31
Upon reaching cruise altitude	1	1.10	0.05	0.08	0.09
Half-way through flight	8	2.35	1.14	0.60	0.10
Just before beginning descent	1	0.58	Undetectable	Uncultivable	0.05
After landing	1	1.90	Undetectable	1.90	0.17
Flight #4					
Pre-Board	4	2.92	0.35	0.24	0.10
Upon reaching cruise altitude	4	5.02	1.61	1.16	0.18
Half-way through flight	3	2.74	1.47	0.66	0.15
Just before beginning descent	2	1.69	0.80	0.11	0.09
After landing	2	6.45	4.23	0.16	0.51

[1]All values are per 100 L of air

[2]Not tested

other samples analyzed, may be the result of inhibitory effects of lavatory cleaning chemicals on the enzymes partaking in the ATP assay. Further research is necessary to validate this claim. The range of total microbial burden, inclusive of both dead and live microbes, was roughly the same between the two airliners tested (10^3 to 10^5 per 100 liters of air).

Lavatory air samples were laden with at least an order of magnitude more microbes than samples collected from all other locations, except for flight #2 aboard Airline A where seat and floor samples were an order of magnitude more burdened than lavatory samples. In contrast, gasper samples were consistently less laden with microbes than samples collected from any other locations. With the exception of flight #1, samples yielded consistent patterns of increasing and decreasing bioburden when plotted against the time of the flight. Flight #1 exhibited a pattern high in microbial burden upon pre-board sampling followed by decreasing burden over the ~2 hour flight period. However, an accumulation of microbes upon reaching cruise altitude and a subsequent decrease in microbial numbers mid-flight was observed for flights #2 and #4. This might be due to the cleaning system employed by the aircraft maintenance crew but no such details were available for this study.

By enzymatically degrading ATP associated with dead microbial cells and other extraneous sources, the ATP arising from both cultivable and non-cultivable microbes can be elucidated (Venkateswaran et al., 2003). Total viable microbial populations were extremely low in both flights of Airline A compared to the samples collected from Airline B flights (Table 1). A threshold of 100 RLU was established to strengthen the conclusions drawn from this data. Only 5 to 15 % of the samples collected aboard Airline A yielded significant RLU values, as 3 of 20 samples analyzed from flight 1, and 1 of 20 samples analyzed from flight 2 had RLU values above the threshold. Airline B was considerably more laden, as 10 of the 14 samples examined on flight 3 and 10 of the 15

Table 2. Percentage of various types of microbes and the cultivable microbial diversity of various air samples collected from several locations of commercial airlines

Description of the samples	Percentage of microbial population that are:			Bacterial species isolated and identified
	viable (B/A*100)	cultivable (C/B*100)	Gram-negative (D/A*100)	
Flight #3				
Pre-Board	33.3	22.1	10.9	*A. johnsonii*
Upon reaching cruise altitude	4.5	160.0	8.2	*A. johnsonii; Oxalobacter* sp.; *Acinetobacter* sp.
Half-way through flight	48.5	52.6	4.3	*A. johnsonii*
Just before beginning descent	Undetectable	Uncultivable	8.6	
After landing	Undetectable	N/A[1]	8.9	*A. johnsonii; Rhodococcus erythropolis* ; *Variovorax* sp.
Flight #4				
Pre-Board	11.8	70.9	3.6	*Microbacterium testaceum*
Upon reaching cruise altitude	32.0	71.8	3.6	*A. johnsonii; Acinetobacter* sp.
Half-way through flight	53.5	44.9	5.5	*Pseudomonas synxantha; Methylobacterium* sp.
Just before beginning descent	47.5	13.9	5.3	*Pseudomonas synxantha; Methylobacterium* sp.
After landing	65.5	3.8	7.8	*Methylobacterium* sp.

[1]Since some tests yielded below detectable levels, ratios were not obtained

examined from flight 4 (~ 70% of the Airline B samples) yielded RLU values above the threshold. In examining the resulting RLU values across several samplings, microbes appear to accumulate from the time of boarding, through reaching cruise altitude, and into early mid-flight, a phenomenon attributed to the respiration of passengers. Furthermore, viable microbes (intracellular ATP) were less abundant in the late mid-flight time period, suggestive of an in-flight recirculation event.

The ratio of the viable population to the total microbial population (Table 1 and 2) in 3 of the 4 Airline A samples that showed detectable intracellular ATP was between 50% and 70%. A ratio of >50% viable microbes to the total microbial burden was observed in 28% of Airline B samples (7 out of 25 samples). The elevated incidence of viable microbes in the cabin air might not be due to the maintenance of the system, rather the exhalation of traveling passengers. Data comparing the number of travelers over a given time frame and maintenance schedule in that time period must be carefully analyzed before drawing any conclusions. In the interest of security, such information was not made available to the authors.

(ii) LPS-targeted endotoxin assay. The results of the LPS-based assay, measured in picograms of endotoxin per 100L of air, are given in Tables 1. As with the results of the ATP assay, LPS analysis revealed that samples collected at varying times and locations aboard Airline B were consistently more laden with LPS than their equivalents collected aboard Airline A. However, the percentage of Gram-negative microbes among the total microbial population was >8% in Airline A, whereas Gram-negatives accounted for <5% of the Airline B burden. The LPS assay yielded highly variable results across both airlines. In general, the collective average of all temporally distinguishable samples arising from the lavatories aboard both aircraft unexpectedly yielded the lowest LPS values. A mere 1 to 6% of the total microbial burden of the lavatory samples appears to be attributeable to Gram-negative bacteria. One sample collected prior to boarding Flight #3 (Airline B) suggested the presence of > 160% more Gram-negative microbes than the total microbial population. This anomaly may be attributable to ATP extraction techniques somehow underestimating total burden and needs to be addressed in future studies.

Table 3. Comparison of 16S rRNA gene-based Q-PCR assay with ATP-assay in the determination of total microbial population[1]

Sample number	Description of the air samples pooled	Number of samples pooled	Q-PCR based 16S rRNA gene copy numbers		ATP (RLU)		Remarks
			Average	SD	Total	Intracellular	
Flight #1							
XXIV	Gasper samples collected during cruise altitude, mid flight and pre-descent	3	1.56×10^4	$\pm$ 5.43×10^3	1.1×10^4	Undetectable	Both methods detected similar signals
XXI	Lavatory samples collected during cruise altitude, mid flight and pre-descent	3	4.97×10^1	$\pm$ 1.08×10^1	8.0×10^4	3.6×10^4	DNA extraction might have failed, since appreciable quantity of intracellular ATP present
XXV	Seat floor samples collected during cruise altitude, mid flight and pre-descent	3	7.69×10^1	$\pm$ 4.38×10^1	1.2×10^4	Undetectable	Higher ATP signal might have derived from non-biological source such as food and human somatic cells
Flight #2							
XXII	Seat floor and Seat height samples collected during pre-board	2	8.09×10^1	$\pm$ 2.16×10^1	1.5×10^4	Undetectable	Higher ATP signal might have derived from non-biological source such as food and human somatic cells
LXIX	Lavatory samples collected during Pre-board, cruise altitude, mid flight, pre-descent, and after landing	5	1.02×10^6	$\pm$ 2.34×10^5	1.1×10^4	Undetectable	Presence of detritus oligotrophs having less cellular metabolism?
XXX	Seat floor and Seat height samples collected after landing	2	1.01×10^4	$\pm$ 7.41×10^3	1.5×10^4	Undetectable	Both methods detected similar signals
ML	Seat Floor samples collected during cruise altitude, mid flight and pre-descent	3	3.90×10^6	$\pm$ 1.84×10^6	1.4×10^4	5.0×10^3	Presence of multi-copied oligotrophs having less cellular metabolism yielding less ATP signals?
Flight #3							
XX	6 Seat height samples collected during interval mid flight at a 30 minutes interval.	6	3.16×10^7	$\pm$ 3.36×10^6	1.3×10^4	4.2×10^3	Very high gene copy numbers (~3 logs higher) could not be explained
Flight #4							
XXIII	Seat Floor samples collected during pre-board and mid flight	2	3.22×10^5	$\pm$ 8.23×10^4	4.9×10^4	1.2×10^4	1-log higher Q-PCR microbial burden might be due to the multi-copy numbers of 16S rRNA gene exisit in microbes
XXVI	Lavatory samples collected during Pre-board, cruise altitude, mid flight, pre-descent, and after landing	5	6.42×10^5	$\pm$ 1.41×10^5	3.4×10^4	4.6×10^3	1-log higher Q-PCR microbial burden might be due to the multi-copy numbers of 16S rRNA gene exist in microbes

[1]All values are per 100 L of air

(iii) TaqMan quantitative PCR. This assay was performed on ten samples generated by pooling multiple spatially-conserved air-impinged samples. Initial attempts at extracting DNA from individual sample failed to yield PCR amplified products. As the ATP analysis shows, many samples housed a mere 10^4 cells per 100 L of air. Hence, samples representing a given location collected at several time points were combined. A description of the pooled air samples and their constituent individual samples is given in Table 3. Pooled samples ranged in total volume, from the combination of 2 to 6 individually collected air-impinged samples. In general, the average 16S rRNA gene copy numbers were in the range of 10^1 to 10^4, 10^1 to 10^6, 10^7, and 10^5 gene copies per 100 L of air for the Flight #1 through 4, respectively. These values could feasibly overestimate or underestimate the microbial population by an order of magnitude, since there are 1 to 10 16S rRNA gene loci per bacterial chromosome. As the total ATP assay predicts the total microbial burden present in a given sample, it seems rational to directly compare the results of this assay with those of the Q-PCR assay.

Results of such comparisons for the 10 pooled samples are depicted in Table 3. As was observed with all other molecular methods, Q-PCR results indicated that Flight #1-borne sample were the least laden with microbes. A detailed analysis of the tested samples has been categorized as follows: The first category consists of samples that yielded similar levels of microbial burden, as estimated by both assays (sample #1 and #6). The second category houses samples that showed ~ 3-logs higher ATP-based microbial burden than that derived via Q-PCR. The ATP content of these second category samples might have originated from non-microbial sources, such as food and human somatic cells (sample #3 and #4). A third category consisted of samples that exhibited 1 to 2-logs higher Q-PCR based microbial burden; the low ATP concentrations measured here might be attributed to the reduced metabolic activity of these microbes (sample #5 and #7). In the fourth and final category, samples presenting a 1-log higher Q-PCR-derived burden are most likely a consequence of the presence of microbes that have multiple16S rRNA gene loci (sample #9 and #10). Molecular microbial diversity analysis should be conducted to confirm this hypothesis. As with any environmental microbiological analysis (Venkateswaran et al., 2003), outliers were common in this study. Sample #2 contained a mere $5.0x10^1$ 16S rRNA gene sequences, this low microbial burden might reflect DNA extraction failure, since an appreciable amount of intracellular ATP was detected in this sample ($3.6x10^4$ cells per 100 L of air). In contrast, the very high gene copy numbers (~3 logs) observed in sample #8 could not be explained.

Culture-dependent analysis.

(i) Plate count assay. Plate count assay results for samples collected from 2 flights aboard Airline B are shown in Table 1. Collectively, the number of colony forming units (CFU) arising from 100L of air ranged from 0 to 4.2×10^4 amongst the 29 samples analyzed. Gasper air samples, thought to be the "cleanest" air about the aircraft, yielded high CFU numbers from both flights. The order of magnitude increase in CFU between gasper samples collected pre-flight and during flight most likely reflects the build up of passenger-borne, respiratory microbes over the course of the flight. The possible accumulation of microbial biofilms about the surfaces of the gasper conduits could also be responsible for such an observation, as the flux of air passing through these channels increases as the aircraft reaches cruise altitude. Given their small, enclosed space, lavatory samples were expected to be very highly laden with microbes, this was not the case as such parcels yielded a mere 3×10^1 to 2×10^3 CFU/100L of air. As was seen with the gasper air samples, lavatory air parcels generally gave rise to more CFU as the flight progressed. The economy class cabin air samples collected from the seat and floor exhibited a great variance in numbers of cultivable microbes (between 0 and 2.7×10^4 CFU/100L air). The trend of increasing microbial burden as the flight progressed was expected, but was not observed in these samples. One plausible explanation for the absence of such a trend is intermixing and circulation throughout the cabin, however, if this were the case it should also hold true for the gasper samples. A more thorough understanding of the airflow dynamics about the cabin at varying times during the flight is required to better understand these observations.

(ii) Ratio of cultivable microbes among total viable microbes. When numbers generated from the plate count assay (total cultivable microbes) were compared with those from the intracellular ATP assay (total viable microbes), about 45% (4 out of 9) and 18% (2 out of 11) of samples from Flight # 3 and #4 respectively, exhibited a higher cultivable population than was suggested by ATP-based analysis (data not shown). This supported the notion that many microbes were metabolically inactive, thus maintaining lower concentrations of ATP. In general, samples from both flights began to accrue cultivable microbes upon reaching cruise altitude followed by a decline in cultivables as the journey progressed (Table 2). This could be due to fluctuating accumulations of certain types of microbes, or induction of a non-cultivable phenotype triggered by pressure-related stress; an in-depth investigation is required before any cogent conclusions into this matter can be drawn. Of the total population, between 2.4% and 24% was cultivable for Flight #3 samples, and this ratio ranged from 1.6% to 80% (7 out of 11 samples) for Flight #4 samples. The results imply that cultivable microbes, such as those collected at sampling events prior to boarding, may become noncultivable upon reaching cruise altitude. Certain pressure-related stresses may be inducing a viable-but-non-cultivable state in these microbes, as has been described previously (Colwell, 2000).

(iii) Identification and diversity of isolates. The identities and overall diversity of the cultivable population present in these samples is given in Table 2. Representatives of the α-, β-, and γ-proteobacteria, as well as Gram-positive bacteria, were identified in varying abundance. Colonies of *Acinetobacter* sp. were by far the most profuse, as made evident by morphometric comparison with sequence identification data. The presence of the opportunistic human pathogens *A. johnsonii* and *A. calcoaceticus* is disturbing, as these members of the Neisseriaceae family can cause meningitis, septicemia, and a

plethora of sometimes fatal diseases and infections (Hunt et al. 2000; Chang et al, 2000; Bergogne-Berezin and Joly-Guillou, 1985). Though beyond the scope of this study, attempts at culturing microbial populations from these samples with a greater variety of media is recommended, and in fact planned for the near future.

Microbial examination of commercial airline cabin air sampled at various times and locations about the cabin, while in flight, revealed that current methods for filtering and purifying cabin air need improvement. The air purification systems on-board the aircraft are allowing the circulation of viable microbial cells of opportunistic pathogens. Additional purification is recommended for the recycling of air throughout the cabin of the aircraft.

CONCLUSION

Perhaps the most apparent trend observed in this study was the tendency of sample locations to accrue microbial burden from the time of boarding through mid-flight, followed by a gradual diminishing of such airborne microbes from late in mid-flight until the time of deplaning. It is also evident that the cabin air aboard Airline A was much less laden with airborne microorganisms than that of Airline B. Such research is paramount for the development and implementation of biological sensors aboard aircraft. In order to train and calibrate sensing devices to detect a particular group of microorganisms the background microbial population must be thoroughly defined.

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